method comprising analyzing nucleic acid of said mammal to determine whether said nucleic acid contains a mutation in a FREAC3 gene, wherein the presence of said mutation is an indication that said mammal has an increased likelihood of developing a disease of the eye, and said FREAC3 gene encodes a polypeptide that is 90% identical to SEQ ID NO: 2.

D1 Cont.

15. (Amended) The method of claim 1, wherein said developmental defect is a cardiac defect or an eye defect, wherein said eye defect is anterior segment dysgenesis.

D2

16. (Amended) The method of claim 1, wherein said mammal is prenatal.

D3

17. (Amended) The method of claim 1, wherein said mammal is a human.

Add E1

REMARKS

The Invention

The present invention relates to novel mutations in the *FREAC3* gene and provides methods for identifying individuals having an increased likelihood of developing certain developmental defects or diseases of the eye.

Support for Amendments

Support for the amendments to claim 1 is found in the specification at page 8, lines 14-18, and page 14, lines 15-23. No new matter is introduced by these amendments.

A "marked up" version of the claims showing the changes made and an appendix of the claims as pending are attached.

The Office Action

Claims 1-5, 8, 11, and 15-17 are pending in this application. All pending claims stand rejected under 35 U.S.C. § 112, for an inadequate written description, a lack of enablement and indefiniteness. Claims 1, 8, 11, 15, and 17 are further rejected under either 35 U.S.C. § 102, as being anticipated by or, in the alternative, 35 U.S.C. § 103, as being obvious in view of Mears *et al.* (Am. J. Hum. Genet. (1996) 59: 1321-1327) and Mirzayans *et al.* (Am. J. Hum. Genet. (1997) 61: 111-119).

Rejections Under 35 U.S.C. § 112, first paragraph

Claims 1-5, 8, 11, and 15-17 are rejected under 35 U.S.C. § 112, first paragraph, for both an inadequate written description and a lack of enablement. Each basis for rejection will be dealt with separately.

Written Description

Claims 1-5, 8, 11, and 15-17 stand rejected under 35 U.S.C. § 112, first paragraph, for an inadequate written description. Specifically, the Examiner states that, although the description of nucleotide and amino acid sequence of the human FREAC3 gene is adequate, claims encompassing mutated and variant sequences, and those from other species are not adequately described. Applicants have amended claim 1, from which the rejected claims depend, and provide the following remarks.

First, Applicants note that claim 1, from which all other rejected claims depend, has been amended to require the FREAC3 gene encode a polypeptide that is 90% identical to the human FREAC3 protein. Accordingly, the amended claim describes a detailed chemical structure of the encompassed nucleic acids. Such nucleic acids were both envisioned and reduced to practice at the time of filing. The specification provides support for FREAC3 genes which are substantially identical to the human(see, for example, page 14, lines 15-23). Additionally, discussed in more detail below, specific

mutations, truncations and a mammalian homolog are described.

Contrary to the Examiner's assertion, Applicants describe the FREAC3 gene from more than one mammalian species, and identify several important mutated and variant sequences. As noted by the Examiner, the nucleotide and amino acid sequence of the human FREAC3 gene are specifically described in SEQ ID NOs: 1 and 2. However, Applicants, in several instances, also identify Mf1 as the murine homolog to FREAC3 (see for example, page 10, lines 12-14; page 40, line 20; and page 42, line 18), and provide a method for the artisan to retrieve the sequence information (page 42, lines 20-22).

Applicants identify key structural and functional domains, and directly compare these domains between species. The forkhead domain of FREAC3, amino acids 69-178, is specifically identified in Figure 2 and at page 43, line 10. Applicants also make several critical comparisons that help a skilled artisan define the metes and bounds of mammalian FREAC3 homologs (page 42, line 11, through page 43, line 1). The human FREAC3 gene and the mouse Mf1 gene are described as 89% identical in their nucleotide sequence through the coding region. The 330 nucleotides that encode the forkhead domains have the greatest degree of identity, 96%. The entire human FREAC3 and murine Mf1 proteins are 92% identical, but 100% identical in the forkhead DNA binding domain. Additionally, helix 1 of the forkhead domain is further characterized by structure and function. Ser82 is identified as an absolute determinant for any FREAC3 homolog to have biological activity, and is conserved in all forkhead family members from diverse species (page 44, lines 1-2). An isoleucine at residue 87 is found in 88% of forkhead genes. Both Ser82 and Ile 87 of helix 1 are believed to reside in the DNA binding domain (page 44, lines 16-21). Thus, taken together, a skilled artisan can envision a detailed chemical structure and many identifying characteristics of the claimed invention.

Turning to the description of mutated sequences and variants of the FREAC3 gene, Applicants note that several examples of both silent and functional mutations are

provided. Three functional mutations of the FREAC3 gene have been identified from human patients. These mutations include G245C and G261C, resulting in S82T and I87M, respectively, and deletion of bp 93-102, causing a frameshift mutation and premature stop. A pair of silent insertion mutations are also identified from human subjects. GGC375ins and GGC347ins each result in a glycine insertions which do not appear to increase the likelihood of disease. Because these insertions were found in both patients and controls, they can be considered as allelic variants of the wild-type human FREAC3 gene.

Accordingly, contrary to the Examiner's assertion, Applicants have described the human and murine FREAC3 gene, and identified several structural and functional domains characteristic of the gene family. Additionally, Applicants have described two allelic variants and numerous functional mutations resulting from insertions, deletions, missense and frameshift mutations. Applicants respectfully submit that the invention, as claimed, is adequately described and this rejection should be withdrawn.

Enablement

Claims 1-5, 8, 11, and 15-17 stand rejected under 35 U.S.C. § 112, first paragraph, for a lack of enablement. Specifically, the Examiner states that, based on Applicants disclosure, a person of ordinary skill in the art could not practice the invention without performing undue experimentation. Four factors are identified for which the Examiner finds the enablement to be insufficient. The factors are: (i) the quantity of experimentation necessary; (ii) the lack of working example; (iii) the unpredictability of the art; and (iv) the lack of sufficient guidance relative to the claim breadth. Applicants respectfully disagree with each of these asserted deficiencies.

Quantity of Experimentation

The court in *In re Wands* defines the boundaries of undue experimentation and states that "the test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine." 858 F.2d 731, 737 (Fed. Cir. 1988). Applicants submit that it is nothing more than routine to isolate DNA from a human subject and analyze a particular gene. DNA is most commonly isolated from blood samples; however, many other sources, including biopsy samples and mucosal scrapings, are often used. As the Examiner points out, the genetic analysis can be done by any one, or a combination of techniques known in the art. It is routine to analyze genetic alterations by genetic sequencing, RFLP, or nucleic acid hybridization techniques. Furthermore, the skill in art of performing these techniques is very high. Many practitioners have M.D.s or Ph.Ds, and there are many commercially available "kits" and other resources for performing these analyses. Therefore, the actual DNA isolation and analysis from a human subject does not constitute undue, or even a large quantity of routine, experimentation.

Working Examples

The Examiner also asserts that the specification lacks working examples. Although, Applicants note that the absence of working examples is not determinative on the issue of enablement (*In re Long* 368 F.2d 892 (C.C.P.A. 1966), the Examiner's assertion is incorrect. Applicants have discovered the linkage between mutations in FREAC3 and human disease by analyzing DNA from human patients. Applicants research has identified three disease-causing mutations (page 19, lines 16-21), including a 10 base pair deletion (nt 93-102), and two missense mutations (G245C and G261C). These mutations are not prophetic examples; they are mutations identified in actual patients suffering from anterior segment dysgenesis.

Predictability of the Art

In further support of this rejection, the Examiner asserts that the art of nucleic acid analysis and the art of predicting enzyme function from structural determinants is unpredictable. Applicants submit that skill in the art of nucleic acid analysis was extremely high at the time of filing. Sambrook *et al.* (1989), the Examiner's own reference that was published nine years before the earliest priority of the present application, demonstrates that a many of the nuances of nucleic acid analysis were appreciated. For example, Sambrook *et al.*, extensively describe mathematical formulae to estimate the kinetics of hybridization and provide guidance on reaction conditions and reagents. In sum, the art of nucleic acid hybridization and analysis was very predictable and the skill in that art was extremely high.

The Examiner also cites Skolnick et al. (2000) for the proposition that the prediction of catalytic function is unpredictable from only structural determinants. The instant specification enables an artisan to determine the function of a mutated FREAC3 polypeptide without relying on a prediction based solely on primary sequence. As discussed in more detail below, in addition to identifying three inactivating mutations found in actual human patients, the specification provides at least two other methods for determining FREAC3 functionality. These alternative methods include: (i) assessing the DNA binding capacity of a FREAC3 polypeptide, and (ii) using transgenic animal technology to determine whether the mutated FREAC3 polypeptide causes an increased likelihood of developing anterior segment dysgenesis.

Guidance Provided by the Specification

In finding that an artisan would be required to perform undue experimentation to practice the invention as claimed, the Examiner states that the claims do not identify relevant nucleic acids, or apprise the skilled artisan that which constitutes a FREAC3 gene. Consequently, the artisan would have to discover the FREAC3 gene sequences,

gene products, and methodologies sufficient to identify normal and mutated sequences. Applicants disagree.

The courts have held that "it is the person in ordinary skill in the field of the invention through whose eyes the claims are construed." *Haynes International, Inc. v. Jessop Steel Co.* 8 F.3d 1573 (Fed. Cir. 1993). Applicants also note that the claims must be read in conjunction with the specification. The meaning of the claim term "a FREAC3 gene" is defined in the specification at page 8, line 14, through page 9, line 1, and is further clarified by the definition of a "wild-type FREAC3." Specifically, the FREAC3 gene is genomic DNA that encodes a FREAC3 polypeptide, and the wild-type is the form most commonly found in a population that is not associated with disease phenotype. Furthermore, mutant FREAC3 is defined as deviation from the wild-type sequence sufficient to confer an increased risk of developing anterior segment malformations (page 9, lines 2-19). The specification also discloses a segment of genomic DNA which contains the FREAC3 coding sequence (SEQ ID NO: 1, Figure 2), and the wild-type FREAC3 amino acid sequence (SEQ ID NO: 2, Figure 2).

The specification further defines the analysis of the FREAC3 to be sufficient to determine whether a FREAC3 gene is wild-type or mutant (page 11, lines 21, through page 12, line 1). As the Examiner notes in the Office Action, the skilled artisan recognizes a multitude of experimental techniques sufficient to analyze nucleic acid sequences (Office Action, page 5). Therefore, it is only left to the artisan to determine whether a mutation is functional or silent.

The specification also enables the artisan to assess the functionality of a FREAC3 mutation. Applicants define functional FREAC3 as biologically active, and sufficient to prevent anterior segment dysgenesis. Applicants define biological activity, and provide a strategy for its determination, at page 9, line 20, through page 10, line 11. Specifically, biological activity may be determined by at least two methods. First, a comparison between the nucleic acid or amino acid sequence of an individual to the known wild-type

(and mutant) FREAC3 sequences. The specification has identified several of the most common mutations in FREAC3 associated with anterior segment dysgenesis, as well as a number of silent mutations that do not cause disease. Additionally, mutations that result in truncated proteins are noted, and well recognized, as being particularly deleterious.

Second, the specification identifies the consensus DNA binding site of FREAC3 as being aGTAAA(T/c)AAAca), and teaches that a reduction in FREAC3 binding to this sequences is indicative of a functional mutation. FREAC3 binding can be quantified by numerous techniques known by those skilled in the art, and identified in the specification. Methods include binding assays, and functional assays using a chimeric gene that operably link the FREAC3 binding site to a reporter gene.

In sum, the specification enables a skilled artisan to practice the invention with nothing more than routine experimentation required. Nucleic acid analysis from human subjects is routine, and three examples of mutated FREAC3 sequences, from actual patients, are disclosed. The skill in the art of nucleic acid analysis is very high, and the techniques used to identify and characterize FREAC3 mutations is routine. The specification also provides several methods for determining whether an altered FREAC3 sequence will result in a functional defect, or will be silent. Accordingly, Applicants respectfully submit that the invention, as claimed, is adequately enabled and this rejection be withdrawn.

Rejections Under 35 U.S.C. § 112, second paragraph

Claims 1-5, 8, 11, and 15-17 are rejected under 35 U.S.C. § 112, second paragraph, as being indefinite with regard to the terms "FREAC3 gene" and "mutations in FREAC3 genes."

The claims have been amended to specify the nucleotide sequence that defines the human FREAC3 gene. Specifically, the claims are limited to genes that encode polypeptides which are 90% identical to human FREAC3, as disclosed in SEQ ID NO: 2.

As presently claimed, a skilled artisan has structural and functional definitions of the products. The specification defines a FREAC3 gene as encoding a wild-type or mutant FREAC3 polypeptide, having normal, or reduced biological activity, respectively (see, for example, page 8, line 14, through page 10, line 11). Therefore, the combination of these functional criteria, with a the requirement for 90% sequence identity to the human FREAC3 gene, clearly define the metes and bounds of the claimed invention. Accordingly, the rejection can be withdrawn.

Rejections Under 35 U.S.C. § 102(b)

Claims 1, 8, 11, 15, 17 are rejected under 35 U.S.C. § 102(b), as being anticipated by Mears *et al.*, (Am. J. Hum. Genet. (1996) 59: 1321-1327) and Mirzayans *et al.*, (Am. J. Hum. Genet. (1997) 61: 111-119). Specifically, the Examiner states that both Mears and Mirzayans teach autosomal dominant mutations that map to 6p25 and that, although both publications fail to disclose a FREAC3 gene or mutation, a skilled artisan would have recognized them as such. Applicants respectfully traverse this rejection.

Far from anticipating a mutation in the FREAC3 gene, Mears merely provides the artisan an invitation for further experimentation. Mears, in studying iridogoniodysgenesis anomaly (IGDA), suggests that an autosomal dominant mutation exists distal to D6S477 at 6p25 and, through linkage studies, maps the IGDA locus to an 8.3 cM inverval (see abstract). An 8.3 cM fragment of genomic DNA corresponds roughly to 8,300,000 nucleotide base pairs (Exhibit A). Watson et al., pioneers in the field of molecular biology, note that cloning a gene 5 cM away from a genetic marker is not trivial (Exhibit A).

Furthermore, in addition to FREAC3, several other genes, including protease inhibitor-6 (placental thrombin inhibitor), coagulation factor XIII, and mitochondrial malic enzyme map to 6p25. Mears does not definitely associate any particular gene present within this region with IGDA. Nor does Mears disclose any mutations in the

genes of 6p25.

Mirzayans does not significantly refine the teachings of Mears. Mirzayans, like Mears, studied genomic DNA from IGDA families using Genomic-Mismatch Scanning. Mirzayans does not identify any particular 6q25 gene by name or sequence, and is only able to extend the teachings of Mears by suggesting that the IGDA locus is contained within a 6.9 cM region flanked by D6S344 and D6S477. This further refinement of the findings of Mears, to approximately 6,900,000 base pairs, does not significantly aid a skilled artisan in identifying the IGDA gene or the specific, disease-causing, mutations.

By contrast to the teachings of Mears and Mirzayans, the FREAC3 gene identified in the present invention encodes a polypeptide in a single exon of 1662 nucleotides. This corresponds to approximately two one hundredths of one percent (0.02%) of the genomic sequence identified by Mears. Additionally, Applicants have identified structural and functional domains of the nucleic acid, as well as allelic variants and inactivating mutations.

Mears and Mirzayans cannot anticipate the present invention because neither reference links any disease of the anterior segment with the FREAC3 gene. The teachings of these two references merely invite further experimentation to identify the disease-causing gene and the specific mutation responsible. Accordingly, the present invention is not anticipated by either Mears or Mirzayans and respectfully request withdrawal of this rejection.

CONCLUSION

Applicants submit that the claims are in condition for allowance, and such action is requested. Enclosed is a petition to extend the period for replying for three months, to and including December 28, 2001. If there are any charges or any credits, please apply them to Deposit Account No. 03-2095.

Respectfully submitted,

Date: November 30, 2001

Kristina Bieker-Brady, Ph.D.
Reg. No. 39,109

Clark & Elbing LLP
176 Federal Street
Boston, MA 02110
Telephone: 617-428-0200
Facsimile: 617-428-7045

\\Clark-w2k1\documents\07540\07540.020003 Reply to OA of 6.28.01.wpd

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Claims as Pending

1. (Twice amended) A method of diagnosing a mammal for an increased likelihood of having a developmental defect or developing a disease of the eye, said method comprising analyzing nucleic acid of said mammal to determine whether said nucleic acid contains a mutation in a FREAC3 gene, wherein the presence of said mutation is an indication that said mammal has an increased likelihood of developing a disease of the eye, and said FREAC3 gene encodes a polypeptide that is 90% identical to SEQ ID NO: 2.

2. The method of claim 1, wherein said mutation is a missense mutation.

3. The method of claim 2, wherein said mutation results in a truncated protein.

4. The method of claim 1, wherein primers are used for detecting said mutation.

5. The method of claim 1, wherein said analyzing includes detecting the loss of a recognition site for a restriction endonuclease.

8. The method of claim 1, wherein said mammal is a human.

11. The method of claim 1, wherein said disease of the eye is glaucoma.

15. (Amended) The method of claim 1, wherein said developmental defect is a cardiac defect or an eye defect, wherein said eye defect is anterior segment dysgenesis.

16. (Amended) The method of claim 1, wherein said mammal is prenatal.

17. (Amended) The method of claim 1, wherein said mammal is a human.

Version With Markings to Show Changes Made

1. (Twice amended) A method of diagnosing a mammal for an increased likelihood of having a developmental defect or developing a disease of the eye, said method comprising analyzing nucleic acid of said mammal to determine whether said nucleic acid contains a mutation in a FREAC3 gene, wherein the presence of said mutation is an indication that said mammal has an increased likelihood of developing a disease of the eye, and said FREAC3 gene encodes a polypeptide that is 90% identical to SEQ ID NO: 2.

15. (Amended) The method of claim 1 [or 7], wherein said developmental defect is a cardiac defect or an eye defect, wherein said eye defect is anterior segment dysgenesis.

16. (Amended) The method of claim 1 [or claim 7], wherein said mammal is prenatal.

17. (Amended) The method of claim 1 [or claim 7], wherein said mammal is a human.

EXHIBIT A

Recombinant DNA

SECOND EDITION

James D. Watson
COLD SPRING HARBOR LABORATORY

Michael Gilman
COLD SPRING HARBOR LABORATORY

Jan Witkowski
BANBURY CENTER, COLD SPRING HARBOR LABORATORY

Mark Zoller
GENENTECH, INC.

SCIENTIFIC
AMERICAN
BOOKS

Distributed by
W. H. Freeman and Company
New York

DNA samples are available for all 4 grandparents in each of the 29 families in the reference panel. Lymphoblastoid cell lines are used for preparing DNA, which is then distributed to members of the CEPH Collaboration. The Collaboration is an international affair with over 60 participating laboratories throughout the world; the members are committed to contributing their linkage data to a CEPH database.

Linkage is Calculated from Frequency of Recombination

Remember that an RFLP is not a mutation that causes a genetic disease. Rather, an RFLP is used as a marker for a gene of interest; that is, it must be linked closely to the gene (Figure 26-5). When pairs of homologous chromosomes come together during meiosis, they exchange segments in a process called *recombination.* The farther an RFLP is from a gene, the more chance there is that there will be recombination between the gene and the RFLP. In a linkage analysis, the coinheritance of marker and gene are followed within a family. The probability that their observed inheritance pattern could occur by chance alone (that is, that they are completely unlinked) is calculated. The calculation is then repeated assuming a particular degree of linkage, and the ratio of the two probabilities (no linkage versus a specified degree of linkage) is determined. This ratio expresses the odds for (and against) that degree of linkage, and because the logarithm of the ratio is used, it is known as the *logarithm of the odds,* or *lod, score.* For practical purposes, a lod score equal to or greater than 3 is taken to confirm that gene and marker are linked. This represents 1000:1 odds that the two loci are linked. Calculations of linkage became much easier with the development of computer programs that were able to perform the many calculations necessary to take into account all the members of large pedigrees. These programs can perform multipoint analyses that calculate linkage between a large number of loci and produce a map of their order along the chromosome.

It is important to remember that markers are placed on a genetic map relative to each other, the order being determined by the recombination between them.

The unit of recombination is the *centimorgan* (cM), named for the great geneticist T. H. Morgan. Two markers are one centimorgan apart if they recombine in meiosis once in every 100 opportunities that they have to do so. The centimorgan is a genetic measure, not a physical one, but a useful role of thumb is that 1 cM is equivalent to approximately 10^6 bp. This relationship between centimorgans and base pairs is a dramatic illustration of the wide gap that exists between genetic and molecular studies. Geneticists consider a marker 5 cM from a gene to be useful for practical purposes such as genetic counseling, and yet the molecular biologist is still 5×10^6 bp away from the gene that is to be cloned! A great deal of research is devoted to developing techniques that are capable of manipulating very large DNA fragments in order to bridge this gap between genetic and physical distances (Chapter 29.)

Recombination has serious consequences when linkage analysis is used to trace the inheritance of a mutation in a family. The association between marker and gene will be lost if the crossover occurs in the region between the marker and the gene, and a misdiagnosis will result (Figure 26-6a). The accuracy of following a gene using RFLPs as markers can be increased substantially if informative RFLPs are available on each side of the gene. In this case a crossover can be detected because the pairing of flanking RFLPs on each chromosome is lost (Figure 26-6b). A double crossover between the flanking markers would not be detected, but if the RFLPs are less than 5 cM from the gene, the chance of a double crossover is 1 in 400 or less. Flanking markers are also important for cloning, because the investigator knows that the gene must lie between them.

Abnormal X Chromosomes Provide a Means of Cloning the Gene for Duchenne Muscular Dystrophy

Both linkage analysis and chromosomal abnormalities were used in the cloning of the gene for Duchenne muscular dystrophy (DMD), an X-linked disorder causing progressive muscle degeneration in young boys. There is no cure, and patients die in their late